Online Supplement

of the manuscript entitled

Pirfenidone in idiopathic pulmonary fibrosis: A phase III clinical trial in Japan

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MATERIALS AND METHODS

Predetermined protocol for definite UIP and probable UIP

Definite UIP pattern was defined by basal predominant, subpleural reticular abnormality with traction bronchiectasis and honeycomb cysts and without atypical features of UIP [E1-3]. Probable UIP pattern was defined as the same as definite UIP pattern, but without apparent honeycombing.

Stepwise manner for treatment regimen of pirfenidone

We used 200 mg tablet of pirfenidone and placebo tablet which is a "dummy" treatment that appears as identical as possible to the test treatment with respect to physical characteristics. During the study, (including dose ascending period), each subjects in the three groups took the same counts of pirfenidone and/or placebo tablet .

Specific details of step-up process are as follows 1) first step up process: in placebo and low dose groups; one 200 mg tablet (study medication– pirfenidone or placebo) was administered to low dose group and one placebo tablet was administered; (*t.i.d.*, equivalent to 600 mg/day within the first step-up process), 2) second step-up process, one 200 mg tablet and one placebo tablet were administered to low dose group (*t.i.d.*, 600 mg/day), and two placebo tablets were administered to placebo group. In the remaining 48 weeks, two 200 mg tablets and one placebo tablet were administered to low dose group (*t.i.d.*, 1200 mg/day), and three placebo tablets were administered to placebo group.

Drugs not allowed to use during this study

This included azathioprine, cyclophosphamide, d-penicillamine, methotrexate, cyclosporine, tacrolimus, interferons and other experimental agents under investigation for IPF.

The procedure of 6-minute steady-state exercise test (6MET)

Patients were requested to walk on a treadmill at a constant speed while breathing air with continuous monitoring of SpO_2 using a pulse-oximeter [E4, E5]. The speed for each patient for the 6MET was adjusted between 30 m to 80 m/minute based on the individual patient's comfort and tolerance to demonstrate > 5% difference between resting SpO_2 and the lowest SpO_2 during 6MET, but without desaturating to < 85% while breathing air at baseline. This predetermined speed at baseline was kept constant throughout the study. During follow up visits, patients were asked to stop walking before the intended duration of the 6MET when the SpO_2 declined to 82% in the interest of their safety.

Assay for KL-6, SP-D and SP-A

KL-6 was measured by Electro Chemiluminescent Immunoassay method. SP-D and SP-A were measured by EIA method.

The sample size estimation

The sample sizes of 100 for the High-dose and Placebo groups were determined based on simulations that would provide statistical power of 0.8 to detect assumed differences of the mean changes in the lowest SpO_2 from baseline to Week 52 between the two groups at a significance level of 0.1 (two-sided). The assumed differences in the two classes of patients with baseline lowest SpO_2 equal to or lower than 88% and upper than 88% were 1.5% (s.d.=4.0%) and 1.0% (s.d.=4.0%), respectively. We simulated baseline and Week 52 observations having 2-variate normal distributions that satisfy the assumptions for High-dose and Placebo groups in the two classes. Then, we set the correlation between baseline and Week 52 observations to be 0.6. We gradually increased the number of simulated observations to explore the sample size that gives the statistical power of 0.8. The sample size of 50 for the Low-dose group was obtained by halving the sample size of 100 for the High-dose and Placebo groups.

RESULTS

Table E1. Comparison of the Change in the PFTs and Pneumocyte Markers

	High-dose		Low-dose		Placebo		p-value#		
	n	mean	n	mean	n	mean	High-dose vs Placebo	Low-dose vs Placebo	High-dose vs Low-dose
TLC	99	-0.16	52	-0.06	99	-0.20	0.5344	0.0408	0.1250
DL_{CO}	96	-0.88	51	-0.51	98	-1.36	0.2317	0.0768	0.4379
PaO_2	98	-2.09	54	-3.39	103	-3.85	0.2433	0.7996	0.4710
$AaDO_2$	98	2.14	54	3.16	103	3.59	0.3325	0.8081	0.5709
KL-6	105	23.97	54	-1.20	104	117.21	0.2228	0.2017	0.7854
SP-D	105	-25.14	54	-18.63	104	-21.90	0.8223	0.8514	0.7090
SP-A	105	1.02	54	0.49	104	-0.89	0.6283	0.7724	0.9122

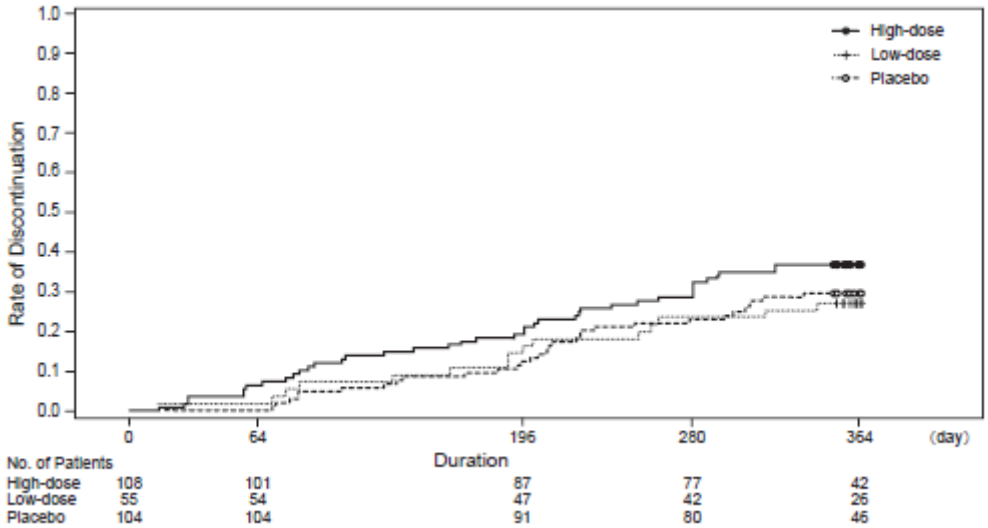
Table E2. Details on patients found to have worsened by status of worsening and by group.

Worsening * \ Group	High-dose	Low-dose	Placebo
1. Death	0	0	1
2. 10% decline in vital capacity	37	21	54
3. Could not be determined due to worsening*	3	0	4
Total	40	21	59

*: The event occurred first was counted for cases experienced more than one events.

Table E3 ' Crude Means ' of VC at each of the observational points: FAS

Week	Group	N	Mean	S.D.	90□ C.I.	
					lower	upper
Baseline	High-dose	106	2.40	0.64	2.30	2.50
	Low-dose	55	2.44	0.68	2.28	2.59
	Placebo	104	2.47	0.70	2.36	2.59
Week 4	High-dose	105	2.43	0.62	2.33	2.53
	Low-dose	54	2.43	0.69	2.28	2.59
	Placebo	103	2.45	0.71	2.34	2.57
Week 8	High-dose	104	2.42	0.64	2.31	2.52
	Low-dose	54	2.44	0.71	2.28	2.60
	Placebo	103	2.45	0.71	2.34	2.57
Week 12	High-dose	98	2.41	0.63	2.31	2.52
	Low-dose	52	2.43	0.75	2.26	2.61
	Placebo	100	2.41	0.72	2.29	2.53
Week 16	High-dose	94	2.43	0.67	2.31	2.54
	Low-dose	49	2.43	0.73	2.25	2.60
	Placebo	98	2.44	0.71	2.32	2.56
Week 20	High-dose	92	2.41	0.64	2.30	2.52
	Low-dose	50	2.40	0.77	2.22	2.58
	Placebo	95	2.44	0.72	2.32	2.56
Week 24	High-dose	91	2.42	0.67	2.30	2.53
	Low-dose	48	2.40	0.75	2.21	2.58
	Placebo	95	2.40	0.71	2.28	2.52
Week 28	High-dose	87	2.40	0.68	2.28	2.52
	Low-dose	47	2.40	0.76	2.22	2.59
	Placebo	93	2.40	0.71	2.28	2.53
Week 32	High-dose	79	2.39	0.69	2.26	2.52
	Low-dose	44	2.37	0.73	2.18	2.55
	Placebo	84	2.42	0.70	2.30	2.55
Week 36	High-dose	77	2.37	0.70	2.24	2.51
	Low-dose	44	2.36	0.75	2.17	2.55
	Placebo	82	2.43	0.71	2.30	2.56
Week 40	High-dose	76	2.39	0.72	2.25	2.53
	Low-dose	42	2.32	0.73	2.13	2.51
	Placebo	79	2.42	0.75	2.27	2.56
Week 44	High-dose	68	2.37	0.71	2.22	2.51
	Low-dose	41	2.33	0.69	2.15	2.51
	Placebo	76	2.43	0.76	2.29	2.58
Week 48	High-dose	66	2.38	0.71	2.24	2.53
	Low-dose	40	2.30	0.66	2.13	2.48
	Placebo	74	2.44	0.77	2.29	2.59
Week 52	High-dose	67	2.36	0.73	2.21	2.51
	Low-dose	38	2.34	0.71	2.14	2.53
	Placebo	72	2.42	0.75	2.28	2.57



Log-rank test (p-values): High-dose group vs Placebo group (p=0.2055),
Low-dose group vs Placebo (p=0.7886), High-dose group vs Low-dose group (p=0.2131)

Figure E1 Kaplan-Meier plot of time to discontinuation

Drop-out rates in High-dose, Low-dose and Placebo groups were 37.0% (40/108), 27.3% (15/55) and 29.8% (31/104), respectively, and the rate in High-dose group was the highest. The distributions of the time were compared in pairs among the 3 groups with log-rank test, but no significant differences were seen.

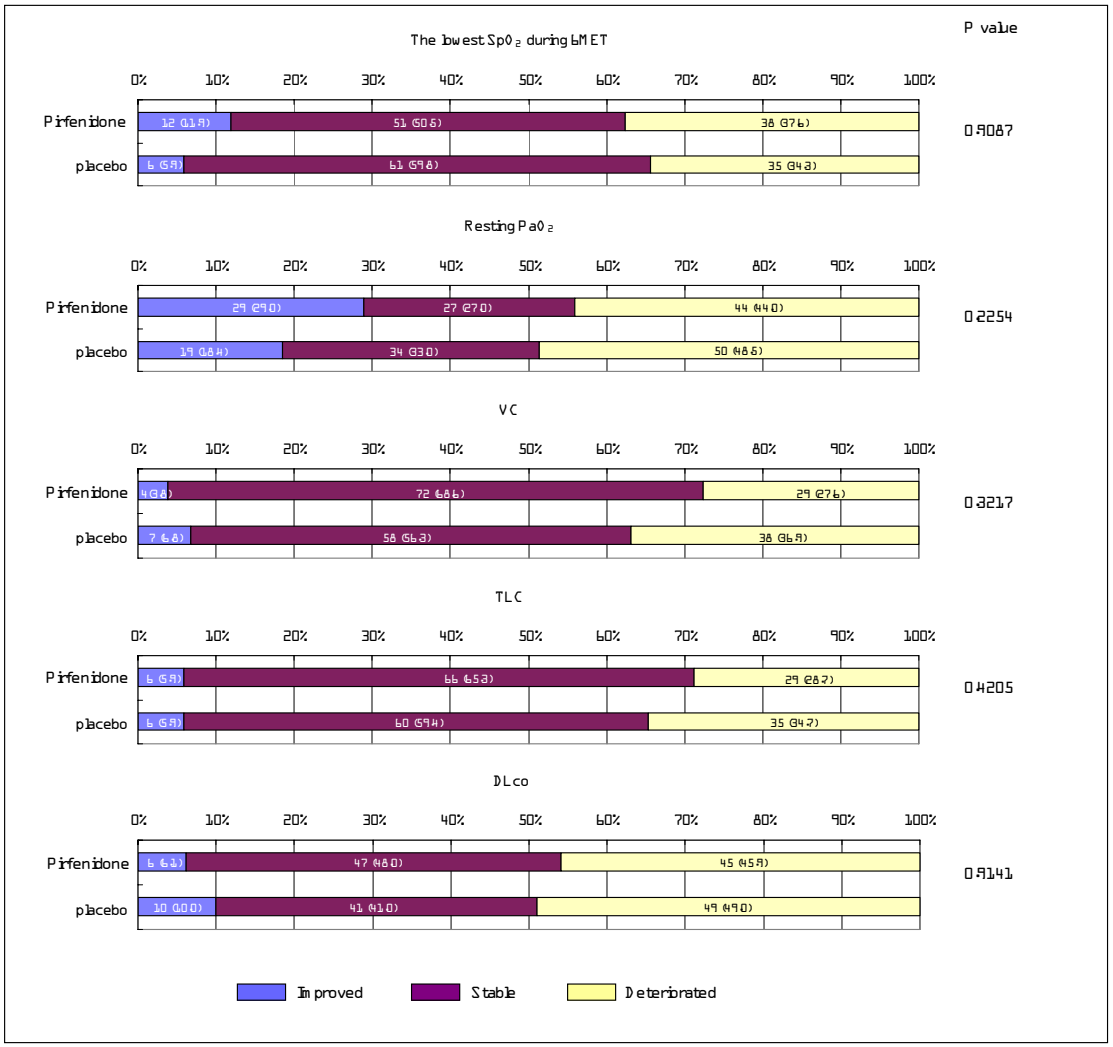


Figure E2-1. Categorized analyses of the lowest SpO_2 during the 6MET and pulmonary functions in the full analysis set (all patients) at 52 weeks.
The results are shown by the categories of improved (blue areas), stable (purple areas), and deteriorated (light yellow areas). P values are indicated at the right. Statistical analysis by Wilcoxon's test was used. Pirfenidone: High-dose group (1800 mg/day).

(Note) Improvement ratings are defined based on ATS criteria as follows:

	respiratory functions			
Ratings	SpO_2	PaO_2	VC & TLC	DLco
Improved	□ 4% increase	□ 4 toll increase	□ 10% increase	□ 15% increase
Stable	< 4% change	< 4 toll change	< 10% change	< 15% change
Deteriorated	□ 4% decrease	□ 4 toll decrease	□ 10% decrease	□ 15% decrease

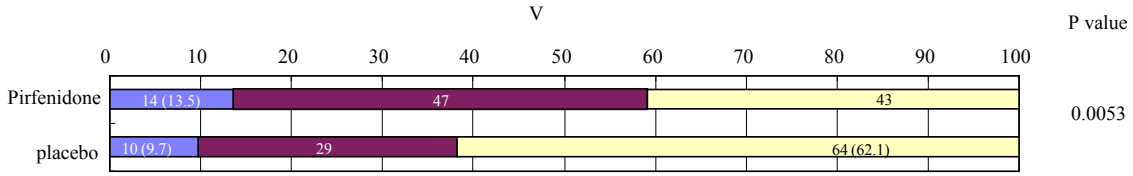


Figure E2-2. Categorized analyses of the changes in VC at 52 weeks based on the rating of a lesser magnitude.

The results are shown by the categories of improved (blue areas), stable (purple areas), and deteriorated (light yellow areas). P values are indicated at the right. Statistical analysis by Wilcoxon's test was used. Pirfenidone: High-dose group (1800 mg/day).

(Note) Improvement ratings are defined based on the reference [29] as follows:

Ratings	VC
Improved	□ 5% increase
Stable	< 5% change
Deteriorated	□ 5% decrease

REFERENCES

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APPENDICES

Japanese Diagnostic Criteria of Idiopathic Interstitial Pneumonias

Translated from "Japanese Guidelines for Diagnosis and Treatment of Idiopathic Interstitial Pneumonias", edited by the Japanese Ministry of Health, Labor, and Welfare Diffuse Pulmonary Disease Research Group (4th edition), published in the Journal of the Japanese Respiratory Society, 2004.

Diagnostic criteria of Idiopathic Interstitial Pneumonias

I. Exclusion of secondary interstitial pneumonias

Exclusion of other known causes of interstitial pneumonia limited to the lung disease such as drug toxicities, environmental exposures, and other diffuse lung diseases and other diffuse lung diseases as listed in Table 1.

II. Primary symptoms, physical findings and examination findings

1. Primary symptoms and physical findings: patients with (1) fine crackles and at least one of the remaining three symptoms are positive:
   (1) Fine crackles
   (2) Dry cough
   (3) Dyspnea on exertion
   (4) Clubbed fingers
2. Serological tests: patients who meet at least one of the following criteria are positive:
   (1) Increased KL-6
   (2) Increased SP-D
   (3) Increased SP-A
   (4) Increased LDH
3. Respiratory functions: patients who meet at least two of the following criteria are positive:
   (1) Restrictive impairment (%VC < 80%)
   (2) Diffusion impairment (%DLco < 80%)
   (3) Hypoxemia (at least one of the following items must be met)

- Resting $PaO_2 < 80$ Torr
- Resting $AaDO_2 \geq 20$ Torr
- SpO_2 during a 6-minute walk test $\leq 90\%$

4. Chest X-ray findings: patients with item 1 and at least one of the remaining items are positive:
   (1) Bilateral diffuse shadows
   (2) Dominant distribution in peripheral region in the middle to lower lung field
   (3) Decreased lung volume
5. Chest HRCT findings: for IPF without pathological diagnosis, items 1 and 2 of the imaging findings below are mandatory. Idiopathic interstitial pneumonias other than idiopathic pulmonary fibrosis show various imaging findings depending on the clinical entity.
   (1) Shadow distribution in the immediately subpleural region
   (2) Honeycombing
   (3) Traction bronchi-bronchiolo-ectasis
   (4) Ground-glass opacity
   (5) Infiltrative shadow (consolidation)

III. Additional reference information for significance of diagnosis:

1. Findings in bronchoalveolar lavage (BAL) are useful in differentiation because they differ with each disease, and thus should be considered as reference. In idiopathic pulmonary fibrosis, cell fractionation is nearly identical to that in BAL from normal lung, with alveolar macrophage being the predominant cell type, but patients with increased neutrophils or eosinophils have poor prognosis. If lymphocytes are increased by 20% or more, a possibility of interstitial pneumonia other than idiopathic pulmonary fibrosis or another disease is suggested, and a treatment response is expected.
2. Transbronchial lung biopsy (TBLB) is not a means for definitive histopathological diagnosis of idiopathic interstitial pneumonias, but provides significant implications as reference or in differential diagnosis (e.g., cancer, granuloma).
3. Surgical lung biopsy by video-assisted thoracoscopic lung surgery (VATS) or by, open lung surgery.

This examination is mandatory for diagnosis of idiopathic interstitial pneumonias other than idiopathic pulmonary fibrosis, and should be used in conjunction with imaging findings for comprehensive diagnosis.

4. Even when the above diagnostic criteria are met, there are cases such as collagen vascular diseases for which the cause of the disease is not revealed until later. In these cases, idiopathic pulmonary fibrosis should be excluded immediately.

IV. Idiopathic pulmonary fibrosis (IPF)

With regard to items 1-5 in **II Primary symptoms, physical findings and examination findings** as described above, patients who correspond to the following definite or probable cases should be diagnosed as IPF:

1. Definite: Patients who meet all of 1-5 in **II**, or are histopathologically diagnosed by surgical lung biopsy as UIP.
2. Probable: Patients who meet 5 and at least two of the remaining items in **II**.
3. Possible: Patients who meet 5 and only one other item in **II**.
4. Idiopathic interstitial pneumonias other than idiopathic pulmonary fibrosis, or another disease: Patients who do not meet 5 in **II**.

V. Idiopathic interstitial pneumonias other than idiopathic pulmonary fibrosis

Patients who are histopathologically diagnosed by surgical lung biopsy, and whose diagnoses are not inconsistent with the clinical, imaging, or BAL findings, etc.

Idiopathic interstitial pneumonias other than idiopathic pulmonary fibrosis include the following diseases:

non-specific interstitial pneumonia (NSIP),

acute interstitial pneumonia (AIP),

cryptogenic organizing pneumonia (COP),

desquamative interstitial pneumonia (DIP),

respiratory bronchiolitis-associated interstitial lung disease (RB-ILD), and lymphocytic interstitial pneumonia (LIP).

VI. Stages of Severity

Stages of severity of idiopathic pulmonary fibrosis are determined according to the severity classification table (Table 2) below. Patients having oxygen partial pressure in resting arterial blood $PaO_2 \geq 80$ Torr are classified as stage I, ≥ 70 Torr and < 80 Torr as stage II, ≥ 60 Torr and < 70 Torr as stage III, and < 60 Torr as stage IV. For patients with stage II or more, if the SpO_2 during a 6-minute walk test is less than 90%, then the severity should be increased by one stage. However, patients whose resting arterial blood gas is less than 70 Torr are not necessarily required for measurement of SpO_2 during a 6-minute walk test.

VII. Diagnostic criteria of acute exacerbation of IPF

1. **Within a month, the following all of the three conditions are satisfied during the disease progress of IPF.**
   - **(1) Dyspnea increases**
   - **(2) New ground-glass opacities appear on HRCT in addition to previous honeycomb lesions**
   - **(3) Oxygen partial pressure in resting arterial blood (PaO_2) is lower by more than 10 Torr than previous one**
2. **Exclude obvious causes, such as infection, pneumothorax, cancer, pulmonary embolism or congestive heart failure**
3. **The serum levels of CRP, LDH are usually elevated as well as serum markers of interstitial pneumonias, such as KL-6, Sp-A or Sp-D**

< Referential information >

In diffuse pulmonary diseases, idiopathic interstitial pneumonia (IIPs) is a generic term referring to interstitial pneumonias with unknown etiology, including idiopathic pulmonary fibrosis (IPF). Classification and diagnosis of IIPs are based on the histopathological diagnosis. However, in clinical settings, surgical lung biopsy, which can provide sufficient information for diagnosis, is often difficult. Therefore, for IPF, which commonly develops in elderly people (predominantly 50 years or older), diagnosis could be made without histopathological investigations if obvious honeycomb lung is confirmed by high-resolution CT (HRCT). If other IIPs are suspected, histopathological diagnosis based on surgical lung biopsy is required.

Table 1. Differential diagnosis of idiopathic interstitial pneumonias

Differential diagnoses requiring exclusion	
(1) Heart failure	(10) Drug-induced pneumonitis
(2) Pneumonia (especially atypical pneumonia)	(11) Eosinophilic pneumonia
(3) Acute lung injury (ALI) with known etiology	(12) Diffuse panbronchiolitis
(4) Tissue connective disease	(13) Lymphangiosis carcinomatosa
(5) Vasculitis	(14) Alveolar cell carcinoma
(6) Sarcoidosis	(15) Pulmonary lymphangioleiomyomatosis (LAM)
(7) Hypersensitivity pneumonia	(16) Pulmonary alveolar proteinosis
(8) Pneumoconiosis	(17) Langerhans cell granulomatosis
(9) Radiation pneumonitis	(18) Others

Table 2. Classification of severity stages of IPF

Severity Stages	Resting PaO_2	SpO_2 during a 6-minute walk test
I	≥ 80 Torr	
II	≥ 70 Torr and < 80 Torr	If < 90%, classified as III
III	≥ 60 Torr and < 70 Torr	If < 90%, classified as IV(if there is a risk, measurement is not required)
IV	< 60 Torr	Measurement is not required